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The contribution of [1H] magnetic resonance spectroscopy to the study of excitation-inhibition in autism

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ABSTRACT

Autism spectrum disorder (ASD) affects over 1:100 of the population and costs the UK more than £32bn and the USA more than $175bn (£104bn) annually. Its core symptoms are social and communication difficulties, repetitive behaviours and sensory hyper- or hypo-sensitivities. A highly diverse phenotypic presentation likely reflects its etiological heterogeneity and makes finding treatment targets for ASD challenging. In addition, there are no means to identify biologically responsive individuals who may benefit from specific interventions. There is hope however, and in this review we consolidate how findings from magnetic resonance spectroscopy (MRS) add to the evidence that differences in the brain's excitatory glutamate and inhibitory γ-aminobutyric acid (GABA) balance may be both a key biomarker and a tractable treatment target in ASD.

1. Introduction

Autism spectrum disorder (ASD) is a highly heterogeneous lifelong neurodevelopmental syndrome characterised by deficits in social reciprocity and communication, and by restricted interests and stereotyped behaviours (DSM-5; American Psychiatric Association, 2013). Its prevalence is growing (Lai et al., 2014) and, in the UK alone, adults with ASD cost the economy over £32 billion/year, i.e. more than heart disease, cancer and stroke combined (Buescher et al., 2014). Therefore, there is a pressing need to identify the underlying biological mechanisms that would provide a basis for the development of ASD stratification markers and treatment options. Progress is happening however, based on accumulating evidence that multiple genetic and environmental risk factors for ASD converge to disrupt the balance between glutamate-mediated excitatory and γ-aminobutyric acid (GABA)-mediated inhibitory neurotransmission; and this may inform treatment targets for the disorder (Nelson and Valakh, 2015; Rubenstein and Merzenich, 2003).

2. Evidence for excitation-inhibition imbalance in autism spectrum disorder

A balanced interaction between glutamate and GABA is essential for synaptic maturation, refinement of neuronal circuitry and subsequently the regulation of cognition, emotion and behaviour (Lujan et al., 2005). An imbalance in excitation-inhibition (E-I) caused by disruption to any component of the glutamate and GABA signalling systems could therefore have potentially far-reaching consequences. Indeed, based on observations such as an increased incidence of epilepsy – a disorder of E-I balance – in individuals with ASD (Bolton et al., 2011; Muhle et al., 2004), and post-mortem evidence of altered expression of proteins involved in glutamate and GABA biosynthesis and neurotransmission in the brains of individuals with ASD (Blatt and Fatemi, 2011; Fatemi et al., 2002, 2009, 2014; Oblak et al., 2009, 2010, 2011; Purcell et al., 2001; Shimmura et al., 2013; Yip et al., 2007), E-I imbalance has been suggested to be a 'common final pathway' in ASD (Nelson and Valakh, 2015; Rubenstein and Merzenich, 2003).

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Genetic studies are in line with this proposal. For example, studies of ASD susceptibility genes have repeatedly identified chromosome 15q11-13 as a particular region of interest as 1–3% of all ASD cases were reported to be associated with duplications in this region (Moreno-De-Luca et al., 2013; Urraca et al., 2013). This chromosomal region contains genes for three $GABA_A$ receptor subtype genes (i.e. β3, γ3 and α5). Mutations in this region, especially in the β3 subunit, convey a high risk for ASD and have been linked to repetitive symptoms and insistence on sameness (Chaste et al., 2014; Isles et al., 2016; Shao et al., 2003). Direct evidence for the link between $GABA_A$-β3 and ASD has also been generated from a β3 knock-out mouse. These mice have behaviours analogous to the condition; they also have seizures, which are a frequent comorbid complication of ASD caused by E-I imbalance (DeLorey et al., 1998). In addition, we and others have reported that environmental risk factors for ASD, such as prenatal exposure to maternal inflammation or the anti-epilepsy medication valproate, disrupt gene and protein expression across GABA pathways and trigger behavioural features which mimic the human condition (Fatemi et al., 2008; Fukuchi et al., 2009; Giovanoli et al., 2014; Meyer et al., 2011; Wei et al., 2016).

There is also good evidence for genetic abnormalities in glutamatergic signalling pathways in ASD and related conditions. This has, in part, been built on the advances made in fragile X syndrome, the most common genetic form of ASD, which is caused by a single gene mutation affecting fragile X mental retardation protein (FMRP) (Verkerk et al., 1991). FMRP negatively regulates postsynaptic glutamate receptor synthesis (including metabotropic glutamate receptor 5; mGluR5) by binding to ribosomes and stalling translation of target mRNAs (Darnell et al., 2011). mRNA translation at the synapse is then increased (Bear et al., 2004), leading to excess excitatory activity, enhanced long term depression, and ASD-like pathology in animal models (Lozano et al., 2014; Yu and Berry-Kravis, 2014). Lower levels of FMRP are found in individuals with idiopathic ASD (i.e. ASD without a known cause) who have a concomitant increase in mGluR5 (Fatemi et al., 2011; Fatemi and Folsom, 2011). In addition to alterations to metabotropic receptors in ASD, genetic aberrations have also been linked to ionotropic glutamate receptors, namely N-methyl-D-aspartate (NMDA) receptors. However, the picture is complex, and both increases and decreases in NMDA receptor function have been associated with the ASD phenotype (Gandal et al., 2012).

GABA and glutamate signalling in ASD may also be indirectly derailed by mutations in genes coding for proteins that stabilise GABAergic and glutamatergic synapses. For example, alterations of neurexin and neuroligin cell adhesion molecules (Ching et al., 2010; Jamain et al., 2003), postsynaptic density protein 95 (PSD-95) and/or Shank scaffold proteins (Uchino and Waga, 2015; Xing et al., 2016) confer a high risk for ASD. These molecules anchor pre- and post-synaptic neuron terminals and disruptions of the molecular assembly of the synapse can potentially cause E-I imbalance (Futai et al., 2007; Südhof, 2008). Consistent with this, neurexin 1-α (*Nrxn1-α*) knock-out mice show a reduction in spontaneous and evoked neurotransmitter release (Kattenstroth et al., 2004; Missler et al., 2003; Zhang, 2005) accompanied by behavioural abnormalities that resemble some of the core ASD symptoms of social impairment and inflexibility/stereotypy (Grayton et al., 2013). Similarly, mice with mutations in the neuroligin 3 gene (*Nlgn3*) exhibit brain-region specific synaptic dysfunction (e.g. a shift of synaptic transmission towards inhibition in the somatosensory cortex and towards excitation in the hippocampus) and impairments in social behaviour that mimic ASD (Burrows et al., 2015; Etherton et al., 2011a, 2011b; Jaramillo et al., 2014; Radyushkin et al., 2009; Tabuchi et al., 2007). Such “multiple” E-I anomalies, that can be not only brain region- but even neural circuit-specific, as shown in multiple animal models of ASD (Horder et al., 2018; Lee et al., 2017; Nelson and Valakh, 2015) further exemplify the complexities of defining and measuring the E-I (im)balance in ASD.

Finally, the metabolism of glutamate and GABA is tightly interlinked so an alteration in either metabolite may potentially affect the other. The enzyme glutaminase catalyses the glutamate to glutamine cycle, allowing glutamine to be stored in astrocytes or converted to glutamate in both glutamatergic and GABAergic neurons (Rowley et al., 2012). In excitatory neurons, glutamate is transported into vesicles via vesicular glutamate transporters, whereas in inhibitory neurons, glutamate is first converted to GABA by the glutamic acid decarboxylase and then transported to vesicles via vesicular GABA transporters. Upon release, the neurotransmitters are taken up by high affinity membrane transporters and returned into neurons and surrounding glia, where they are recycled. Thus, GABA, glutamate and glutamine are in constant flux. In ASD however, the levels of the multiple enzymes controlling glutamine-glutamate-GABA cycles are altered (Fatemi et al., 2002; Shimmura et al., 2013; Yip et al., 2007), and so glutamine-glutamate-GABA metabolism is likely to be atypical in the ASD brain.

How these diverse E-I anomalies might act mechanistically has been the subject of a number of recent comprehensive reviews (Foss-Feig et al., 2017; Gao and Penzes, 2015; Gatto and Broadie, 2010; Lee et al., 2017; Mullins et al., 2016; Nelson and Valakh, 2015; Uzunova et al., 2016). For example, Yizhar et al. (2011) have shown that increase of a cellular E-I balance in the mouse medial prefrontal cortex (mPFC) elicits elevated rhythmic high-frequency (gamma) activity (a disease correlate in clinical conditions; Orekhova et al., 2007) and social dysfunction. Furthermore, it was recently shown that reducing the E-I ratio by either reducing the excitability of pyramidal neurons or by increasing the excitability of inhibitory parvalbumin-positive neurons in the mPFC rescued social deficits in mice lacking the *CNTNAP2* gene, which has been implicated in ASD (Selimbeyoglu et al., 2017). Maintenance of E-I balance is thus essential to network function as not only functional, but also structural properties of neural circuits are defined by inter-neuronal communication.

3. [1H] Magnetic resonance spectroscopy evidence for E-I imbalance in ASD

The hypothesis that the glutamine-glutamate-GABA system is altered in ASD has also been addressed by using [1H] magnetic resonance spectroscopy (MRS) as a non-invasive method to measure tissue levels of E-I metabolites in the living human brain (Ford and Crewther, 2016; Schur et al., 2016). In brief, [1H]MRS uses the nuclear magnetic resonance properties of hydrogen atoms to generate a frequency spectrum (as opposed to the image in MR imaging) (Govindaraju et al., 2000; Juchem and Rothman, 2014; Stagg et al., 2011) in which different metabolites are identified by the position of their signal peak, i.e. chemical shift, along the frequency axis (in units of ‘parts per million’; ppm). [1H]MRS metabolite levels can then be quantified by calculating the area under the corresponding peaks or by fitting the in vivo spectra to a basis set, i.e. linear combinations of measured or simulated model spectra of metabolites (Edden et al., 2014; Naressi et al., 2001; Provencher, 1993). [1H]MRS is not without limitations however. For an adequate signal-to-noise ratio, standard single voxel [1H]MRS data is acquired from a predefined and relatively large brain volume-unit (usually around 4–8 cm^3) (Stagg et al., 2011; Zhu and Barker, 2011) compared with conventional MR image resolution (typically around 1–10 mm^3) (Blüml, 2013). Therefore, both grey and white matter is usually sampled within the same voxel. To avoid errors when quantifying metabolites, these so-called ‘partial volume’ effects need to be corrected by segmenting spectroscopic voxel according to different tissue contents (Dager et al., 2008; Quadrelli et al., 2016). As shown in Table 1, most of the studies reported using tissue segmentation, however, exact approaches varied considerably between studies which might contribute to inconsistencies in the literature. Such partial volume effects are minimised in [1H]MRS imaging (MRSI), a less frequently used extension of [1H]MRS that allows data from multiple adjacent small (1 cm^3 and below) voxels to be acquired simultaneously (Blüml, 2013; Oz et al., 2014; Schneider, 2016; Zhu and Barker, 2011).

Table 1
Summary of [1H]MRS studies reporting Glx, Glu and/or GABA+ levels in ASD.

Citation	ASD group N	Age (years)	[1H] MRS sequence (TR/TE/Av)	Quantification method (tissue segmentation)	Brain areas investigated (cm^3)	Metabolite concentrations in ASD participants compared with controls		
						Glx	Glu	GABA+
Paediatric studies		Range						
Friedman et al. (2006)	45	3–4	1.5 T MRSI PEPSI (2000/20,272/na)	Estimated concentration (Y)	Grey matter (na) White matter (na)	NSD NSD	na	na
DeVito et al. (2007)	26	6–17	3 T MRSI (1800/135/na)	Estimated concentration (Y)	Grey matter (na) White matter (na) Cerebellum (na)	Lower in ASD NSD Lower in ASD	na	na
Hardan et al. (2008)	18	8–15	1.5 T MRSI STEAM (1600/20/4)	Estimated concentration (Y)	(L/R) Thalamus (5.8)	NSD	na	na
Harada et al. (2011)	12	2–11	3 T STEAM (5000/15/48) 3 T MEGA-PRESS (2500/68/256)	Estimated concentration (Y)	(L) Frontal lobe (27) Lenticular nuclei (27)	na	NSD NSD	Lower in ASD NSD
Bejjani et al. (2012)	8 26	7–15 6–17	1.5 T PRESS (1500/25/256) 1.5 T MRSI PRESS (1500/30/8)	Estimated concentration (Y)	(L/R) Anterior cingulate cortex (2.4–3.6) (L/R) Anterior cingulate cortex (1.1)	Higher in ASD Higher in ASD (R)	na	na
Kubas et al. (2012)	12	8–15	1.5 T PRESS (1500/35/192)	Creatine ratios (na)	(L/R) Frontal lobe (na)	Lower in ASD	na	Lower in ASD
Corrigan et al. (2013)	45	3–4	1.5 MRSI	Estimated concentration (Y)	Grey matter (na) White matter (na)	NSD Lower in ASD	na	na
	31	6–7	PEPSI (2000/20/na)		Grey matter (na) White matter (na)	NSD NSD		
	29	9–10			Grey matter (na) White matter (na)	NSD NSD		
Hassan et al. (2013)	10	6–14	1.5 T PRESS (1500/30/3)	Estimated concentration (na)	Anterior cingulate cortex (8) (L) Striatum (8) (L) Cerebellum (8) (L) Frontal lobe (8)	na	Higher in ASD Higher in ASD Higher in ASD Higher in ASD	na
Joshi et al. (2013)	7	12–17	4 T 2D-JPRESS (2000/30–250/16,32)	Estimated concentration (Y)	Anterior cingulate cortex (8) (L/R) Medial temporal lobe (3.38)	na	Higher in ASD NSD	na
Doyle-Thomas et al. (2014)	20	7–18	3 T MRSI (2000/30/na)	Creatine ratios (na)	(L/R) Caudate (na) (L/R) Putamen (na) (L/R) Thalamus (na)	NSD Higher in ASD NSD	na	na
Gaetz et al. (2014)	17	11	3 T MEGA-PRESS (1500/68/256)	Creatine ratios (Y)	(L) Motor cortex (27) (L) Auditory cortex (24) Visual cortex (27)	na	na	Lower in ASD Lower in ASD NSD
Rojas et al. (2014)	17	14	3 T MEGA-PRESS (2500/70/512)	Creatine ratios (Y)	(L) Auditory cortex (36)	na	na	Lower in ASD
Brix et al. (2015)	14	10	3 T PRESS (1500/35/128) 3 T MEGA-PRESS (1500/68/256)	Estimated concentration Creatine ratios (Y)	(L) Anterior cingulate cortex (27)	NSD	na	NSD
Cochran et al. (2015)	13	13–17	3 T PRESS (2000/28/128) 3 T MEGA-PRESS (2000/68/320)	Estimated concentration Total creatine ratios (Y)	Anterior cingulate cortex (8 for PRESS, 18 for MEGA-PRESS)	na	NSD	NSD
Drenthen et al. (2016)	15	14–18	3 T PRESS (2000/35/128) 3 T MEGA-PRESS (2000/68/320)	Creatine ratios (Y)	Occipital cortex (27)	na	NSD	NSD
Naaijen et al. (2016)	51	8–13	3 T PRESS (3000/30/96)	Estimated concentration (Y)	Anterior cingulate cortex (8) (L) Dorsal striatum (8)	na	Higher in ASD NSD	na
Goji et al. (2017)	34	2–12	3 T STEAM (5000/15/48)	Estimated concentration (na)	Anterior cingulate cortex (6 for STEAM, 27 for PRESS)	NSD	na	NSD
	23	4–9	3 T PRESS (2500/68/256)		(L) Cerebellum (6 for STEAM, 27 for PRESS)	NSD		NSD
Hegarty et al. (2017)	47 twin pairs	10	3 T MRSI PRESS (2300/35/na)	Total creatine ratios (Y)	(L/R) Thalamus (4.5)	Lower in ASD (L)	na	na

(*continued on next page*)

Table 1 (*continued*)

Citation	ASD group N	Age (years)	[1H] MRS sequence (TR/TE/Av)	Quantification method (tissue segmentation)	Brain areas investigated (cm^3)	Metabolite concentrations in ASD participants compared with controls		
						Glx	Glu	GABA +
Ito et al. (2017)	112	4–14	3 T STEAM (5000/15/48)	Creatine ratios (na)	Anterior cingulate cortex (6 for STEAM, 27 for MEGA-PRESS)	NSD	NSD	Lower in ASD
	114	3–14	3 T MEGA-PRESS (2500/68/256)		(L) Cerebellum (6 for STEAM, 27 for MEGA-PRESS)	NSD	Higher in ASD	Lower in ASD
Port et al. (2017)	16	6–14	3 T MEGA-PRESS (1500/68/256)	Creatine ratios (Y)	(L) Auditory cortex (24)	na	na	Lower in ASD
Puts et al. (2017)	35	8–12	3 T MEGA-PRESS (2000/68/320)	Estimated concentration (Y)	(R) Sensorimotor cortex (27) Occipital cortex (27)	na	na	Lower in ASD NSD
Cavalho Pereira et al. (2018)	27	9–18	3 T MEGA-PRESS (1500/68/392)	Estimated concentration Total creatine ratios (Y)	Medial prefrontal cortex (27)	NSD	na	NSD
Adult studies		Mean (SD)						
Page et al. (2006)	25	35 (11)	1.5 T PRESS (3000/35/160)	Estimated concentration Total creatine ratios (Y)	(R) Amygdala – hippocampal complex (6) (R) Parietal lobe (8)	Higher in ASD NSD	na	na
Bernardi et al. (2011)	14	29 (6)	3 T MRSI PRESS (2000/30/na)	Estimated concentration (na)	(L/R) Anterior cingulate cortex (0.56) (L/R) Thalamus (0.56) (L/R) Temporo-parietal junction (0.56) (L/R) Intraparietal sulcus (0.56)	Lower in ASD (R) NSD NSD NSD	na	na
Aoki et al. (2012)	24	29 (6)	3 T STEAM (3000/15/128)	Estimated concentration (Y)	Medial prefrontal cortex (8)	NSD	na	na
Brown et al. (2013)	13	36 (6)	3 T PRESS (2000/30/192)	Estimated concentration (na)	(L/R) Auditory cortex (7.5)	Higher in ASD	Higher in ASD	na
Horder et al. (2013)	28	28 (6)	1.5 T PRESS (3000/30/na)	Estimated concentration (Y)	(L) Basal ganglia (6) (L) Dorsolateral prefrontal cortex (7.68) (L) Medial parietal lobe (8)	Lower in ASD NSD NSD	na	na
Tebartz van Elst et al. (2014)	29	35 (9)	3 T PRESS (3000/30/na)	Estimated concentration (Y)	Anterior cingulate cortex (8) (L) Cerebellum (8)	Lower in ASD NSD	Lower in ASD NSD	na
Libero et al. (2015)	19	27 (1)	3 T PRESS (2000/80/128)	Creatine ratios (Y)	Anterior cingulate cortex (5.4) Posterior cingulate cortex (10.8)	NSD NSD	na	na
Robertson et al. (2015)	20	29 (9)	3 T MEGA-PRESS (1500/68/320)	Estimated concentration (Y)	Visual cortex (18.75) (L) Motor cortex (18.75)	NSD NSD	na	NSD NSD
Libero et al. (2016)	20	26 (1)	3 T PRESS (2000/80/128)	Estimated concentration Total creatine ratios (Y)	Anterior cingulate cortex (5.4) Posterior cingulate cortex (10.8)	NSD NSD	na	na
Ajram et al. (2017)	17	33 (2)	3 T MEGA-PRESS (2000/68/na)	Estimated concentration (Y)	Dorsomedial prefrontal cortex (30)	NSD	na	NSD
Endres et al. (2017)	24	40 (10)	3 T PRESS (1500/30/256)	Estimated concentration (Y)	Anterior cingulate cortex (15) (L) Dorsal prefrontal cortex (15.63)	NSD NSD	na	na
Port et al. (2017)	15	21 (1)	3 T MEGA-PRESS (1500/68/256)	Creatine ratios (Y)	(L) Auditory cortex (24)	na	na	NSD
Horder et al. (2018)	25	30(1)	3 T MEGA-PRESS (2000/68/368)	Estimated concentration (Y)	(L) Striatum (26.25) Medial prefrontal cortex (30)	Lower in ASD NSD	Lower in ASD NSD	NSD NSD
Kirovski et al. (2018)	12	28(6)	3 T MEGAPRESS (1500/68/240)	Estimated concentration (Y)	(R) Dorsolateral prefrontal cortex (8) (R) Superior temporal sulcus (8)	na	na	NSD

Estimated concentration refers to water- or external reference-scaled metabolites.

ASD, autism spectrum disorder; Av, number of averages; GABA +, γ-aminobutyric acid + macromolecules; Glx, glutamate + glutamine; Glu, glutamate; L, left; MRS, magnetic resonance spectroscopy; MRSI, magnetic resonance spectroscopy imaging; MEGA-PRESS, Meshcher-Garwood point-resolved spectroscopy; na, not applicable; NSD, not statistically different; PEPSI, proton echo planar spectroscopy imaging; PRESS, point-resolved spectroscopy; R, right; ROI, region of interest; STEAM, stimulated echo acquisition mode; TR, repetition time (ms); TE, echo time (ms); Y, reported.

However, MRSI is often associated with reduced spectral resolution, non-uniform water suppression and voxel bleeding artefact (i.e. signal contamination from neighbouring voxels into the voxel-of-interest), therefore single voxel [1H]MRS remains the technique of choice (Astrakas and Argyropoulou, 2016; Blüml, 2013; Duarte et al., 2012; Jansen et al., 2006).

This large volume also means that [1H]MRS samples tissue metabolites within both neurons and glia and in both intra-and extra-cellular space (Dager et al., 2008). Therefore, to account for possible variation in voxel tissue composition, some studies report metabolite concentrations as ratios to total creatine (creatine + phosphocreatine; tCr), which is assumed to be present at constant concentration in brain tissue and cerebrospinal fluid (Gussew et al., 2012). However, as an alternative to tCr ratios, water has been used as a reference because tCr itself can be altered in neurodevelopmental conditions (Alger, 2010). Finally, depending on technical factors, such as the magnetic field strength, it is not always possible to differentiate between glutamate and glutamine due to the overlap of their resonant peaks, and the combined signal termed Glx is commonly reported (Table 1), and thus is only a proxy measure of glutamate. Similarly, the GABA signal usually also has a contribution from 'macromolecules', and is denoted as GABA+ (Edden et al., 2012; Henry et al., 2001; Mikkelsen et al., 2017; Near et al., 2011).

Despite these limitations, [1H]MRS is safe and therefore, in contrast to other in vivo imaging modalities such as positron emission tomography, it can be used repeatedly, and also in children. Indeed, many [1H]MRS studies of E-I metabolites in ASD have been conducted in paediatric populations. The initial studies focused on the glutamatergic system, reporting Glx and glutamate (Glu), with reduced levels found in the cortical grey matter, frontal lobes, thalamus and cerebellum in children with ASD (DeVito et al., 2007; Hegarty et al., 2017; Kubas et al., 2012) (Table 1). However, this was not replicated by others, with either no difference in Glx/Glu observed between ASD and control participants in cortical and subcortical areas (Brix et al., 2015; Cavalho Pereira et al., 2018; Cochran et al., 2015; Doyle-Thomas et al., 2014; Drenthen et al., 2016; Friedman et al., 2006; Goji et al., 2017; Harada et al., 2011; Hardan et al., 2008; Ito et al., 2017; Joshi et al., 2013; Naaijen et al., 2016) or higher levels reported in ASD – in the anterior cingulate cortex, frontal lobes, striatum (Bejjani et al., 2012; Doyle-Thomas et al., 2014; Joshi et al., 2013; Hassan et al., 2013; Naaijen et al., 2016) and cerebellum (Hassan et al., 2013; Ito et al., 2017) (Table 1).

By definition, ASD is a neurodevelopmental condition and how the brain matures is acknowledged to be quite different to even age matched controls (Amaral et al., 2008; Aoki et al., 2012; Redcay and Courchesne, 2005). Thus, results of [1H]MRS studies in ASD – as other MR modalities – may vary depending on the age group sampled. For example, Corrigan et al. (2013) have reported lower Glx in the cerebral white matter of 3–4-year-old children; however, there was no change in Glx when the same children were scanned again at age 6–7 and 9–10 years; and no difference in grey matter Glx at any age in childhood (Table 1). However, even in adulthood, when the brain may be expected to have fully matured, [1H]MRS studies of glutamate have generated conflicting findings. Lower Glx/Glu has been reported in the anterior cingulate cortex in ASD adults (Bernardi et al., 2011; Tebartz van Elst et al., 2014) (Table 1), but others have observed no significant difference in Glx between ASD adults and controls in this cortical region (Endres et al., 2017; Libero et al., 2015, 2016) (Table 1). Horder et al. (2013, 2018) reported lower Glx/Glu in the basal ganglia of adults with ASD, but unchanged cortical levels (Table 1).

In addition to differences in study samples and brain regions examined, different [1H]MRS sequences for quantification of Glx and/or Glu have been employed in the studies reviewed here (Table 1). While conventional [1H]MRS sequences (e.g. STEAM, PRESS) were used by most, unedited (equivalent to a PRESS) (Horder et al., 2018; Robertson et al., 2015) as well as the difference MEGA-PRESS spectra (edit ON-edit OFF subtraction; please see below) (Ajram et al., 2017; Cavalho Pereira et al., 2018) were also used to quantify Glx and/or Glu (Table 1).

In order to sample GABA, customised spectral editing sequences, such as Meshcher-Garwood Point-resolved spectroscopy (MEGA-PRESS) carried out at 3 T or higher field strengths are required (Mescher et al., 1998; Mullins et al., 2014). In MEGA-PRESS, a GABA signal can be resolved from the more prominent Cr + PCr signal at 3 ppm by applying an editing pulse (edit ON) to the GABA signal at 1.9 ppm interleaved with edit OFF pulse (no frequency-selective editing pulses applied, or if applied, at a different frequency) (Cleve et al., 2015; Long et al., 2015; Maddock et al., 2016; Mescher et al., 1998; Mullins et al., 2014; Shungu et al., 2016). The application of this editing pulse results in the co-editing of other molecules present in the final spectrum, namely the Glx at 3.75 ppm and 'macromolecules' at 3 ppm (Cleve et al., 2015; O'Gorman et al., 2011). Thus, the GABA signal at 3 ppm is commonly reported as GABA+ (Table 1). Furthermore, due to low tissue concentration of GABA, MEGA-PRESS requires larger ROI volumes (typically around 27 cm^3) and longer acquisition times ideally between 7 and 10 min (Mikkelsen et al., 2018). This was generally applied in all but two reviewed study (Kirovski et al., 2018; Kubas et al., 2012) (Table 1). In ASD, most studies of [1H]MRS GABA+ have been conducted only in the last four years (Table 1). The majority have recruited children (Brix et al., 2015; Cavalho Pereira et al., 2018; Cochran et al., 2015; Drenthen et al., 2016; Gaetz et al., 2014; Goji et al., 2017; Harada et al., 2011; Ito et al., 2017; Kubas et al., 2012; Puts et al., 2017; Rojas et al., 2014), with three studies involving only adults (Ajram et al., 2017; Horder et al., 2018; Kirovski et al., 2018; Robertson et al., 2015), and one combining children and adults (Port et al., 2017). The pattern of GABA+ differences in ASD compared to typically developing controls is a little more consistent than for Glx and Glu, with either lower measures or no difference in regional GABA+ reported. Thus, lower GABA+ has been found in the motor, auditory and frontal cortices and in the sensorimotor regions, anterior cingulate cortex and cerebellum (Gaetz et al., 2014; Harada et al., 2011; Ito et al., 2017; Kubas et al., 2012; Port et al., 2017; Puts et al., 2017; Rojas et al., 2014), whereas others have observed no differences in GABA+ in the prefrontal and occipital cortices (Ajram et al., 2017; Brix et al., 2015; Cavalho Pereira et al., 2018; Cochran et al., 2015; Drenthen et al., 2016; Gaetz et al., 2014; Goji et al., 2017; Horder et al., 2018; Puts et al., 2017; Robertson et al., 2015), lenticular nuclei (Harada et al., 2011), motor cortex (Robertson et al., 2015), superior temporal sulcus (Kirovski et al., 2018), basal ganglia (Horder et al., 2018) and cerebellum (Goji et al., 2017) (Table 1).

Reduction in GABA in the somatosensory cortex has been associated with altered tactile function in children with ASD (Puts et al., 2017). Interestingly, developmental tactile perception abnormalities due to a loss of presynaptic inhibition of somatosensory neuron transmission have been shown to result in ASD-related social interaction deficits in mice (Orefice et al., 2016). However, even in the absence of group differences in GABA+, a role for GABA in ASD has still been argued based on findings that [1H]MRS GABA+ levels in ASD are inversely correlated with scores on the Autism Spectrum Screening Questionnaire (Brix et al., 2015) and with the communication and delayed development scores of the Autism Diagnostic Interview – Revised (Cavalho Pereira et al., 2018). More difficult to disentangle are the results from Robertson et al. (2015). In their study, occipital GABA+ measures were equivalent in ASD and control groups, but the ASD group had a deficit in the performance of a binocular rivalry test of visual function thought to rely upon visual cortical E-I balance. The authors went on to show that a positive correlation between GABA+ and performance on a binocular rivalry task in control group was not evident in the ASD group, and suggested that the results supported an impairment in 'inhibitory signalling' in ASD (Robertson et al., 2015). This may well be the case, but because [1H]MRS captures the metabolites within a composite of cell types and does not capture active neurotransmission, it is

impossible to say with certainty.

4. E-I flux in ASD

One perspective on this constellation of [1H]MRS findings in ASD, is that their range may mirror the etiological and phenotypical heterogeneity of the spectrum as well as the variety in the typically developing control groups used for comparison. For example, [1H]MRS measures of glutamate and GABA+ differ even within the typically developing population (Bogner et al., 2010; Brix et al., 2017; Kaiser et al., 2005; O'Gorman et al., 2011; Sailasuta et al., 2008).

Another consideration for the field is that, in the main, indices of glutamate and GABA have been assessed separately in ASD; yet the results have been interpreted in terms of E-I balance (Dickinson et al., 2016). It could be argued that a true imbalance can only be uncovered if the level of each metabolite is reported. Thus, if a GABA decrease is accompanied by a glutamate decrease, then the system may remain balanced. Moreover, until recently, the logic of trying to capture metabolites within a glutamine-glutamate-GABA cycle that is constantly changing or in 'flux' in cohorts with and without ASD, has not been questioned. We now suggest that replication difficulties in cross-sectional studies of glutamate and GABA pathways in ASD arise, at least in part, because they sample only one part of an E-I system, which is a 'moving target'. As described earlier, the regulatory enzymes controlling this dynamic system are altered in ASD, and so E-I flux in ASD is therefore very likely to be atypical.

Supporting this hypothesis, there is now evidence that [1H]MRS is sensitive to changes in E-I flux elicited by pharmacological probes that challenge the system. This comes initially from studies in rats, which have established that [1H]MRS can reliably capture changes in glutamate and GABA levels following pharmacological interventions (Waschkies et al., 2014). Specifically, [1H]MRS was used to track the E-I response to pharmacologically relevant doses of compounds with a known mechanism of action on GABAergic and glutamatergic neurotransmission, namely vigabatrin, 3-mercaptopropionate, tiagabine, methionine sulfoximine, and riluzole. Clear drug dose-[1H]MRS effect relationships were demonstrated, with changes as low as 6% in glutamate and 12% in GABA reliably measured using [1H]MRS. The authors concluded that quantitative [1H]MRS of glutamate and GABA may usefully reveal E-I 'target engagement' – in other words, the interaction of a drug with its biological goal (Waschkies et al., 2014). We have recently extended this approach to humans. Using [1H]MRS, we found that the anti-glutamate and pro-GABA drug riluzole increased the proportion of GABA+ relative to the total level of GABA+ and Glx metabolites in the prefrontal cortex in ASD, but decreased it in controls, despite a comparable baseline between groups (Ajram et al., 2017).

This difference in E-I 'responsivity' in ASD has important implications for the development of pharmacological treatments for ASD, as it suggests that drugs may not act in the ASD brain the same way as in the typically developing brain. That is, selecting a compound for a trial/treatment in ASD based on its mechanism of action in the typical brain may not always be appropriate. Moreover, the response to pharmacological treatments in ASD may itself be heterogeneous. This may, for instance, explain why some – but not all – individuals with ASD can have paradoxical responses to $GABA_A$/benzodiazepine receptor agonists; usually these medications have a sedative effect, but 'excitation' has at times been reported in individuals with ASD (Lemonnier et al., 2012; Marrosu et al., 1987). It may also explain some of the trial disappointments in ASD. For example, lamotrigine, an anticonvulsant, was proposed to be a potential treatment for ASD because it inhibits synaptic glutamate release in excitatory neurons. Unfortunately, it did not improve clinical outcomes in children with ASD (Belsito et al., 2001). A similar failure followed the use of D-cycloserine, a partial agonist at the NMDA glutamate receptor (Belsito et al., 2001; Minshawi et al., 2016; Posey et al., 2004).

5. Conclusions

We conclude that disruption within E-I systems could serve as a biomarker for ASD. However, we extend this observation to suggest that E-I systems in the ASD brain are pharmacologically atypical. However, the picture is far from straight-forward. This means that we need better strategies, potentially including [1H]MRS, to examine the biological response or target engagement to proposed treatments in sub-groups of individuals with ASD. The identification of more pharmacologically homogeneous sub-groups within the spectrum may inform a new generation of biologically-driven clinical trials and avoid the time and expense (and failure) of un-stratified large-scale studies.

Author contributions

L.A.A., M.M.P., and G.M.M. designed the study. L.A.A., A.M.S.D., A.C.P., H.E.V., M.M.P., and G.M.M. wrote the manuscript. All authors contributed to and approved the final manuscript.

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Declarations of interest

None.

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